Proton pumping in cytochrome *c* oxidase: The coupling between proton and electron gating

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Cytochrome *c* oxidase is the terminal oxidase in cellular respiration. This membrane protein accepts electrons from ferrocytochrome *c* in the periplasmic space of the mitochondrion, one electron at a time, and transfers the reducing equivalents to the binuclear heme-iron copper site (the so-called Fe_{a3}, Cu_B site), where dioxygen binds and the O·O bond is subsequently cleaved (1). In this manner, the binuclear center is activated by the dioxygen, and the heme iron is oxidized by two oxidizing equivalents to form Fe_{a3}–oxyferryl, namely, $Fe_{a3}^{4+} = O^{2-}$, and the Cu_B site by two oxidizing equivalents to form the Cu_B^{2+}–OH^-/tyrosyl radical species (2). Subsequent inputs of additional reducing equivalents from reduced cytochrome *c* to the low-potential Cu_A and Fe_a centers and the transfers of these electrons to the activated binuclear center are linked to proton pumping (3).

In a high-resolution X-ray structural analysis of the CO, NO, and CN^- derivatives of bovine heart cytochrome *c* oxidase reported in PNAS, Yoshikawa et al. (2) have attempted to derive insights into the possible structural changes that might occur in the enzyme near the active site upon the activation of the binuclear center by molecular oxygen. It is conjectured that dioxygen binding to the Fe_{a3}^{2+} first generates a Fe_{a3}^{3+}-superoxo species (Fe_{a3}^{3+}-O_2^-), mimicking a CN^- species bound to the oxidized Fe_{a3}^{3+} (Fe_{a3}^{3+}–CN^-). Subsequent transfer of two reducing equivalents from the Cu_B^{1+} and Tyr244, the tyrosine that is covalently linked to the Cu_B histidine ligand His240, cleaves the O·O bond, creating the oxylferryl species ($Fe_{a3}^{4+} = O^{2-}$) and the oxidized Cu_B site (Cu_B^{2+}–OH^-/tyrosyl radical) mentioned earlier. The authors argue that this mechanism of dioxygen reduction minimizes the likelihood of releasing active oxygen species, which is undoubtedly true. More importantly, however, the authors have inferred from these structural results a set of conformational changes at the binuclear site that might be linked to the gating of the protons poised for proton pumping, an obligatory step in the overall mechanism of proton translocation mediated by this enzyme.

A redox-linked proton pump is a complex molecular machine (3–6). First, there must be sufficient redox energy to drive the protons uphill: in the case of cytochrome *c* oxidase, against the protomotive force that exists across the inner membrane of the mitochrondrion. To accomplish the process with reasonable rates, there must be some compromise of the thermodynamic efficiency in the conversion of the redox energy to chemical work. As a kinetically driven process, some fraction of the redox energy driving the proton-pumping reaction mediated by cytochrome *c* oxidase must be dissipated as heat. Second, there must be a molecular mechanism coupling the redox energy driving the process to the protons that are being pumped. In other words, there must be a mechanism of redox linkage (3, 4). This mechanism could be an electrostatic one, in which the coulomb interaction between the electron and proton somehow drives the coupled vectorial proton and electron transfer in opposite directions (Fig. 1). In this simple scenario, the protons must first be gated by dioxygen activation of the binuclear site, which ultimately accepts the electrons linked to the vectorial proton translocation.

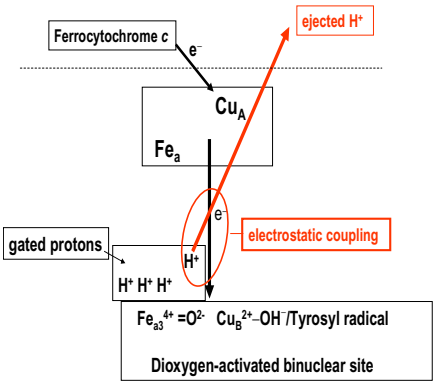

Fig. 1. A simple electrostatic model for coupling uphill proton translocation to downhill electron transfer.

Alternatively (4), with the binuclear site already activated by dioxygen, the redox linkage could occur at Cu_A or Fe_a, the site(s) receiving the reducing equivalents from the cytochrome *c*. Here, part of the redox energy between the low-potential centers and the dioxygen-activated binuclear center could be transferred nonadiabatically to the protein, changing its conformation irreversibly, albeit slightly, but in a manner that gates the electron flow as well as the protons to be pumped (Fig. 2). This gating of the electron flow would allow the enzyme to

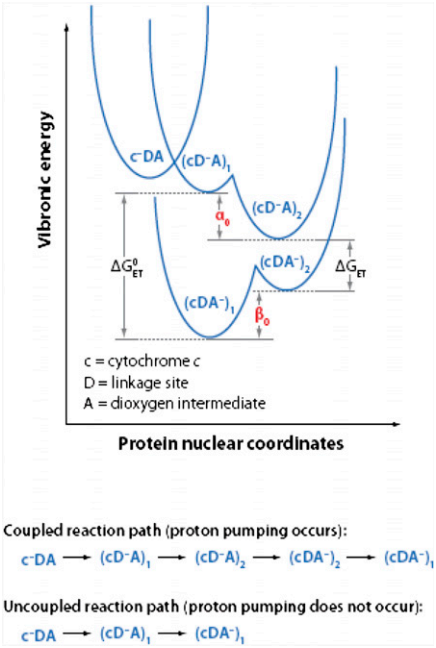

Fig. 2. Schematics of redox linkage occurring during proton pumping by cytochrome *c* oxidase (CcO). In this model, both Cu_A and the dioxygen-activated Fe_{a3} and Cu_B are involved in the redox linkage. The primary electron donor that initiates the process is cytochrome *c*, which is denoted by c. D denotes the electron donor for the electron transfer coupled to the proton pump (i.e., the input linkage site), and A represents the electron acceptor—either the Fe_{a3} oxyferryl dioxygen intermediate or the Cu_B^{2+}–OH^-/tyrosyl radical (i.e., the output linkage site). Proton-pumping and nonproton-pumping conformational states of the CcO complex are highlighted by the subscripts 2 and 1, respectively. These symbols are used in unison [e.g., $(c^-DA)_1$, $(cD^-A)_1$, $(cD^-A)_2$, $(cDA^-)_2$] to denote a specific redox and conformational state of the CcO complex. Plotted along the ordinate is the total vibronic energy of the enzyme complex, the electron donating cytochrome *c*, and the protons involved in the dioxygen chemistry and the proton-pumping process. The abscissa denotes the protein nuclear coordinates. α_0 and β_0 denote the amounts of redox energy transferred to the protein to excite the vibrational degrees of freedom during the redox linkage from the input and output redox linkage sites, respectively. ΔG_{ET} denotes the driving force for electron transfer. This figure has previously been published as figure 2 in ref. 5.

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switch from an "uncoupled" to a "coupled" pathway, accelerating the downhill electron transfer as well as the electron-coupled proton transfer across the osmotic barrier when there is sufficient driving force to drive the process kinetically. If not, the coupled pathway would not be able to compete with the uncoupled pathway, and the outcome is an electron leak.

In principle, both types of scenarios could contribute to the overall redox linkage to drive the vectorial proton translocation in cytochrome *c* oxidase, instead of one or the other of the two limits. Moreover, proton gating could occur during dioxygen activation of the enzyme, and the coupling between the electron and proton could be achieved by electron gating during the downhill electron transfer. Even so, there must still be some conformational change in the protein during the coupling between the electron and the proton that is being pumped to facilitate the movement of the proton across the osmotic barrier. Thus, the distinction between the two models of redox linkage might not be as disparate as it seems above.

Regardless of the model of linking the redox energy to the translocation of the protons across the membrane, Muramoto et al. (2) have offered a molecular proposal for the gating of the protons during proton pumping on the basis of structural changes at the binuclear site incurred by dioxygen activation. This represents an advance in our efforts to understand the mechanism of proton pumping mediated by cytochrome *c* oxidase since the crystal structure of the enzyme was first reported some 15 years ago (7–9).

It seems that the electron gating mechanism is more likely in cytochrome *c* oxidase.

One feature of the proton gating proposed by Muramoto et al. (2) is that all four protons that are ultimately pumped across the membrane are sequestered into a water channel at the outset during the cleavage of the O·O bond to activate the binuclear center. Accordingly, the chemical potential of these protons will decrease (become more negative) with each successive proton ejected into the intermembrane space due to dissipation of the electrostatic energy among the protons sequestered in the water channel with each step. Thus, for a mechanism of proton translocation that is driven by direct proton-coupled electron transfer in opposite directions, the efficacy of the vectorial proton transfer will be highly sensitive to the tightness of the direct linkage between the electron and the proton. If not, molecular slips or proton backflows will prevail. On the other hand, in a mechanism based on electron gating, the efficiency of the proton pumping will decrease with increasing electron pressure and the opposing protomotive force counteracting the proton transfers because uncoupled electron transfers or electron leaks become more prevalent under these conditions. In light of these considerations, it seems that the electron gating mechanism is more likely in cytochrome *c* oxidase because there is always sufficient redox energy to sustain an electron leak in this redox-linked proton pump.

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